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COMMENTARY

Do anthocyanins and anthocyanidins, cancer chemopreventive pigments in the diet, merit development as potential drugs?

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Abstract Anthocyanins, plant pigments in fruits and berries, have been shown to delay cancer development in rodent models of carcinogenesis, especially those of the colorectal tract. Anthocyanins and anthocyanidins, their aglycons, especially cyanidin and delphinidin, have been subjected to extensive mechanistic studies. In cells in vitro, both glycosides and aglycons engage an array of anti-oncogenic mechanisms including anti-proliferation, induction of apoptosis and inhibition of activities of oncogenic transcription factors and protein tyrosine kinases. Anthocyanins and anthocyanidins exist as four isomers, interconversion between which depends on pH, temperature and access to light. Anthocyanidins are much more prone to avid chemical decomposition than the glycosides, and they only survive for minutes in the biophase. These pharmaceutical issues are very important determinants of the suitability of these flavonoids for potential development as cancer chemopreventive drugs, and they have hitherto not received adequate attention. In the light of their robust cancer chemopreventive efficacy in experimental models and their superior stability as compared to that of the aglycons, the anthocyanins seem much more suitable for further drug development than their anthocyanidin counterparts.

Keywords Cancer chemoprevention · Anthocyanins · Drug development

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Introduction

Epidemiological studies suggest that consumption of certain diets, for example fruits, vegetables and fibre, can reduce the risk of developing cancer. Therefore, in recent years, the putative cancer chemopreventive properties of dietary constituents have become a focus of intense scrutiny. The search for efficacious and safe diet-derived alternatives to pharmaceuticals exemplified by aspirin, finasteride and tamoxifen, has been rendered even more relevant in the light of the realisation that diet constituents, in addition to delaying the onset of certain cancers, may also augment the efficacy of chemotherapeutic drugs in the treatment of established disease [1]. The plethora of published studies on mechanisms which may mediate chemopreventive or co-chemotherapeutic effects of dietary constituents contrasts sharply with a dearth of information on their clinical pharmacological and pharmaceutical properties. Whilst such information is vital to help adjudge the suitability of developing them as potential drugs, it tends to attract insufficient attention by researchers in the field. Carried away by the enthusiasm generated by a particularly intriguing biochemical property in cells in vitro of the dietary component under study, they often conclude articles describing the novel mechanism to propose that the intervention in question "…could be developed as agent for the management of cancer…". Such statements are often premature, if not out of place, because pharmacological and

pharmaceutical issues including stability, bioavailability and formulatability of the dietary constituent need to be taken into account before it can, even remotely, be considered for further drug development. This argument is particularly germane in the light of the precarious stability and bioavailability of many polyphenolic phytochemicals. The present review illustrates these issues focusing on anthocyanins, water-soluble pigments which occur abundantly in fruits and berries, and anthocyanidins, their aglycons. It summarizes the evidence for their cancer chemopreventive efficacy and juxtaposes it with their clinical pharmacology and pharmaceutical properties. The objective of the review is to highlight properties of this class of flavonoids which may define generic criteria applicable to the development of dietary agents.

Pharmaceutical properties

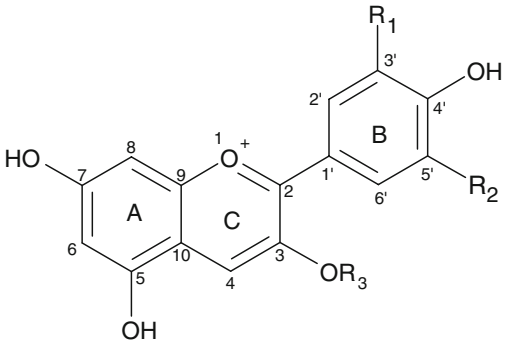
The anthocyanins found most commonly in higher plants are the glycosides of the six anthocyanidins, with percentage abundance in brackets, cyanidin (50%), pelargonidin (12%), peonidin (12%), delphinidin (12%), petunidin (7%) and malvidin (7%) (Fig. 1) [2]. The most common sugar components of anthocyanins are glucose, galactose and arabinose, usually conjugated to the C3 hydroxyl group in the anthocyanidin C ring. To date, in excess of 400 naturally occurring anthocyanins have been identified [2].

The pharmaceutical Achilles heel of anthocyanidins and anthocyanins is their existence as several molecular forms in equilibrium with each other which depends highly on temperature, pH and presence of light and oxygen [3]. At pH <2 a relatively stable flavylium cation predominates, responsible for the intense red colouration (Fig. 2a). Increasing pH is accompanied by rapid loss of a proton generating a blue quinoidal base. Hydration of the flavylium cation yields a colourless carbinol pseudo-base. This species tautomerises, through opening of the C ring, to generate a yellow chalcone (Fig. 2a). Both glycosides and aglycons can spontaneously degrade to a phenolic acid and phloroglucinol aldehyde (Fig. 2b), the latter probably via the intermediate trihydroxyphenyl acetaldehyde, both rather elusive species. The rate of breakdown differs dramatically between anthocyanins and their aglycons (vide infra).

The dosage forms in which anthocyanins have been administered in preclinical rodent experiments and clinical trials have been almost exclusively berry preparations, single agents have been administered in only a few studies.

Pharmacokinetics and metabolism

ADME investigations have focussed almost exclusively on anthocyanin-rich plant products, i.e. naturally occurring mixtures of anthocyanins. Anthocyanins are rapidly absorbed and eliminated. Their bioavailability has been found to be consistently low across animal species including humans (reviewed in [3]). It is important to note two analytical chemical issues which complicate the interpretation of the ADME of anthocyanins and anthocyanidins. Firstly, species not absorbed in the upper gastrointestinal tract can be degraded and/or biotransformed by the gut microflora to furnish small phenolic aldehydes and acids undetectable by the analytical methods usually employed to measure anthocyanins or their aglycons [4]. Secondly, almost all analytical methods which have been used to measure these flavonoids involve acidification of biomatrix samples to transform species present at neutral pH, i.e. colourless carbinol pseudo-base and chalcone forms, into the flavylium cation (Fig. 2a). Metabolic conversion which impedes this conversion would result in failure to detect the analyte [5]. These issues illustrate that the clinical pharmacology of anthocyanins warrants much more study involving



Cyanidin $R_1 = OH, R_2 = H$

Delphinidin $R_1 = OH, R_2 = OH$

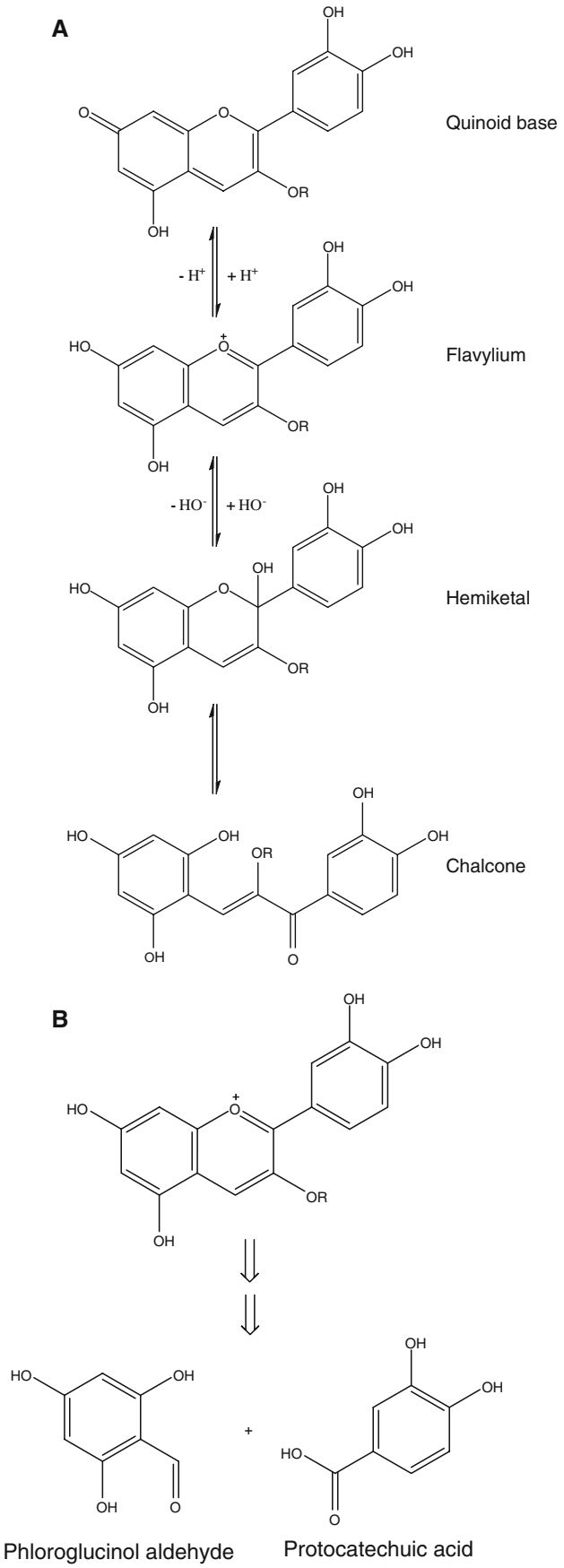
Petunidin $R_1 = OCH_3, R_2 = OH$

Peonidin $R_1 = OCH_3, R_2 = H$

Malvidin $R_1 = OCH_3\ R_2 = OCH_3$

R_3 = Glucose, galactose or arabinose

Fig. 1 Structure of anthocyanidins and anthocyanins



◀ **Fig. 2** Molecular forms (**a**) and decomposition products (**b**) of anthocyanins and anthocyanidins, exemplified by cyanidin glycoside (*R* = sugar) and cyanidin (*R* = H) [37]

novel analytical approaches. Protocatechuic acid, generated from cyanidin-3-glucoside chemically and metabolically in the microflora, has recently been measured in human plasma at concentrations far in excess of the anthocyanin consumed as a constituent of Sicilian blood oranges [6]. However, formation in vivo of protocatechuic acid as a major cyanidin glycoside metabolite seems to be species-dependent, as it was not found in the biophase of rats on cyanidin-3-glucoside [7]. Glucuronidated and methylated conjugates are major metabolites of anthocyanins, and such species have been isolated from human urine and plasma [8, 9]. Aglycones and sulphate conjugates have been identified as minor metabolites in human urine [10–12]. The presence of aglycones has also been demonstrated in jejunal tissue and plasma of rodents on oral anthocyanins [13] and, indirectly, in pigs which consumed a chokeberry extract. In the latter study, identification of cyanidin monoglucuronide in the urine implied intermediate formation of the aglycon [14].

Preclinical evidence for cancer chemopreventive properties

Anthocyanins and anthocyanidins, predominantly in the form of mixtures, have demonstrated cancer chemopreventive properties in animal models of breast, skin, oesophageal, lung, oral and gastrointestinal carcinogenesis (Table 1). Evidence for efficacy is arguably most convincing for the latter, as illustrated by the following examples: supplementation of the diet with 0.3% mirtocyan, a standardised bilberry extract containing 15 anthocyanins, caused about 30% reduction in tumour number and burden in Apc^{Min} mice, a model of human familial adenomatous polyposis [15]. Isolated cyanidin-3-glucoside at that dose was similarly efficacious. This study identified anthocyanin concentrations in the blood and gastrointestinal tract associated with efficacy. An anthocyanin-rich extract derived from tart cherries reduced adenoma multiplicity but not size in this model [16]. In another study, Apc^{Min} mice received either a mixture of anthocyanins or pure cyanidin in their drinking water at 800 mg/l or 200 mg/l, respectively. A third group received dietary supplementation with 20% freeze-dried tart cherries [17]. The number and burden of caecal adenomas was reduced in all mice that received flavonoids compared to those that consumed a control diet, without an effect on small intestinal or colonic adenomas. Anthocyanins have also exhibited chemopreventive

properties in chemically-induced models of colorectal carcinogenesis. Rats received 1,2 dimethylhydrazine (DMH) to induce colorectal adenomas and carcinomas followed by dietary supplementation (at 5%) with extracts of purple corn, purple sweet potato or red cabbage and the carcinogen 2-amino-1-methyl-6-phenylimidazopyridine (PhIP) which induces aberrant crypt foci (ACF) [18, 19].The average number of adenomas and adenocarcinomas was significantly reduced in rats which received flavonoids compared to controls. Induction of ACF by PhIP was significantly inhibited by purple corn and red cabbage colourings. Dietary supplementation with up to 10% lyophilized black raspberries significantly reduced azoxymethane-induced colorectal adenocarcinoma and ACF multiplicity in rats [20]. Anthocyanin-rich extracts of bilberry, chokeberry and grape also inhibited the development of ACF in this model [21]. Whilst all of these data strongly indicate chemopreventive efficacy of anthocyanins in rodent models of carcinogenesis, we know hardly anything about differences in potency in these models between individual anthocyanin molecules.

Clinical evidence for cancer chemopreventive properties

Anthocyanin-rich fruit or berry preparations have been investigated in healthy volunteers or individuals at high risk of developing cancer. Table 2 lists such studies and endpoints germane to cancer chemoprevention. Freeze-dried black raspberry gel was topically applied four times per day to 17 patients with oral intraepithelial neoplasia [22]. After 6 weeks pre- and posttreatment biopsies were evaluated for change in histopathology grade. Seven patients showed histopathological improvement, six exhibited stable disease and four evidence of progression. A reduction in loss of heterozygosity at tumour suppressor gene-associated loci was also observed. Loss of heterozygosity is linked to the development of many human cancers, including oral squamous cell carcinoma. There was an association, although relatively weak, between reduction in loss of heterozygosity and improvement in histopathology grade [22].

Trials of black raspberry powder are currently ongoing in patients with familial adenomatous polyposis who have undergone a subtotal colectomy with ileorectal anastomosis [23]. Over a period of 9 months, patients take two black raspberry powder suppositories daily in combination with an oral dose of 20 g of black raspberry powder or placebo. Endoscopic biopsies are obtained at baseline, 18 and 36 weeks. Preliminary data from 18 weeks suggests that consumption of this powder may be associated with rectal polyp regression. Twenty patients with Barrett's oesophagus have been recruited into a trial of this berry preparation [23, 24]. They consumed up to 45 g of black raspberry powder daily for 6 months. Endoscopic biopsies were obtained before and after treatment. Limited data to date suggests that the length of Barrett's lesions were unaltered by treatment. When urinary markers of oxidative stress were assessed, 8-epi-prostaglandin F2α was significantly reduced, but there was no significant change in mean urinary levels of 8-oxo-7,8-dihydro-2′-deoxyguanosine.

Recently, 25 patients with confirmed colorectal cancer received mirtocyan daily for 7 days before they underwent colectomy or resection of hepatic metastases [25]. Daily dose levels were equivalent to 0.5, 1.0 or 2.0 g of anthocyanins. Mirtocyan consumption caused a small decrease in proliferation and small increase in apoptosis in tumour tissue. Total levels of anthocyanins achieved in the biophase resembled those accompanying efficacious doses in the Apc^{Min} mouse [15] when doses were compared on the basis of body surface.

The case for anthocyanidins as potential drugs

Anthocyanidins possess intriguing anticarcinogenic properties. They inhibit the growth of a range of tumour cells and induce apoptosis [26–33]. Delphinidin and cyanidin have been shown to inhibit the protein tyrosine kinase activity of the epidermal growth factor receptor and downstream events [33–36]. Anthocyanidins bearing methoxy moieties in the B-ring like malvidin interfered with 3′,5′-cAMP-specific phosphodiesterase PDE4 [34]. Delphinidin induced apoptosis and inhibited NFκB signalling in prostate tumour cells in vitro and in a human prostate tumour xenograft in nude mice in vivo [26]. These findings have engendered the suggestion that delphinidin might be considered for development as potential anticancer agent. As intriguing as the mechanistic observations are, the suitability of this or other anthocyanidins for drug development needs to be interpreted in the light of their exquisite chemical instability, which confounds their clinical potential. In cell culture medium, delphinidin and cyanidin broke down with half-lives of less than 30 min, whilst the half-lives of pelargonidin and peonidin were approximately an hour [32]. Furthermore, anthocyanidins generate hydrogen peroxide in cellular incubations, so that their cell growth-inhibitory efficacy in vitro may well have been the combined effect of a complicated mixture of phloroglucinol aldehyde, phenolic acid, hydrogen peroxide and parent compound. The stability of anthocyanidins in plasma and tissues has not been clearly defined, except that they broke down rapidly in the presence of intestinal microflora [37]. Anthocyanidins released locally from their glycosides have been suspected to mediate, at least in part, the efficacy of anthocyanins. Consistent

Table 1 Chemopreventive efficacy of anthocyanins/anthocyanidins in animal models of carcinogenesis

Model	Anthocyanin preparation	Anthocyanin dose (% diet, wt/wt)*	Length of treatment (weeks)*	Primary outcomes
Breast, DMBA-treated rats [42]	Grape juice	Up to 781 mg/L drinking water	20	↓ Tumour incidence weeks 9 and 10[†] ↓ Final tumour mass[†]
Breast, DMBA-treated rats [43]	Grape juice	Up to 830 mg/L drinking water	3	↔ Tumour incidence ↓ Final tumour mass[†] ↓ DMBA-DNA adducts (mammary and liver[†]) ↓ 8-oxo-dG (mammary)[◇] ↔ 8-oxo-dG (liver)
Colorectum, *Apc*^{Min} mice [17]	Tart cherry extract	800 mg/L drinking water	10	↓ Adenoma number and volume (caecum) ↔ Adenoma number (colon/small intestine)
	Cyanidin	200 mg/L drinking water	10	↓ Adenoma number and volume (caecum) ↔ Adenoma number (colon/small intestine)
	Freeze-dried ground tart cherries	0.008–0.02	10	↓ Adenoma number and volume (caecum) ↔ Adenoma number (colon/small intestine)
Colorectum, *Apc*^{Min} mice [15]	Cyanidin-3-glucoside	Up to 0.3	12	↓ Adenoma number (intestine)[†] ↓ M_1dG (adenomas)[◇]
	Bilberry extract	Up to 0.1	12	↓ Adenoma number (intestine)[†] ↓ M_1dG (adenomas)[◇]
Colorectum, *Apc*^{Min} mice [16]	Tart cherry extract	Up to 0.3 + 0.01 sulindac	19	↓ Adenoma number (small intestine)[‡] ↓ Adenoma area (small intestine)[‡] ↔ Adenoma size (small intestine)[‡]
Colorectum, azoxymethane-treated rats [20]	Freeze-dried black raspberries	Up to 0.2	33	↓ Aberrant crypt foci (colon) ↓ Tumour number (colon) ↓ Mean tumour burden (colon)[◇] ↓ 8-oxo-dG (urine)
Colorectum, azoxymethane-treated rats [21]	Chokeberry extract	0.4	14	↓ Aberrant crypt foci (colon) ↔ 8-oxo-dG (urine) ↔ COX-2 mRNA (colon) ↓ PCNA (colon)
	Bilberry extract	0.4	14	↓ Aberrant crypt foci (colon) ↔ 8-oxo-dG (urine) ↓ COX-2 mRNA (colon) ↓ PCNA (colon)
	Grape extract	0.4	14	↓ Aberrant crypt foci (colon) ↔ 8-oxo-dG (urine) ↓ COX-2 mRNA (colon) ↔ PCNA (colon)
Colorectum, azoxymethane-treated rats [44]	Black soybean	0.006	11	↓ Aberrant crypt foci (colon)[◇] ↓ COX-2 mRNA (colon) ↓ PGE_2 (plasma)
Colorectum, DMH-and PhIP-treated rats [18]	Purple corn colour	1.1	32	↓ Aberrant crypt foci (colon) ↓ Adenoma number (colon) ↓ Adenocarcinoma number (colon) ↔ Extra-colonic tumours
Colorectum, DMH- and PhIP-treated rats [19]	Purple sweet potato colour	2.5	32	↔ Aberrant crypt foci (colon) ↓ Adenoma number (colon) ↔ Adenocarcinoma number (colon) ↔ Extra-colonic tumours
	Red cabbage colour	3.0	32	↓ Aberrant crypt foci (colon) ↓ Adenoma number (colon) ↓ Adenocarcinoma number (colon) ↔ Extra-colonic tumours

Table 1 continued

Model	Anthocyanin preparation	Anthocyanin dose (% diet, wt/wt)*	Length of treatment (weeks)*	Primary outcomes
Lung, NNK- and B[*a*]P-treated mice [45]	Freeze-dried strawberries	Not reported	Up to 25	↔ Tumour incidence or multiplicity
Oesophagus, NMBA-treated rats [46]	Freeze-dried strawberries	Not reported	24	↔ Papilloma incidence ↓ Tumour multiplicity ↓ O^6-methylguanine adduct (oesophagus)
Oesophagus, NMBA- treated rats [47]	Freeze-dried strawberries	Not reported	Up to 32	↔ Tumour incidence ↓ Tumour multiplicity ↓ O^6-methylguanine adduct (oesophagus)
Oesophagus, NMBA-treated rats [48]	Freeze-dried black raspberries	Not reported	Up to 35	↓ Tumour incidence$^{\diamond}$ ↓ Tumour multiplicity ↔ Tumour size ↓ O^6-methylguanine adduct (oesophagus)†
Oesophagus, NMBA-treated rats [49]	Freeze-dried blueberries	Not reported	Up to 25	↔ Tumour incidence ↔ Tumour multiplicity ↓Tumour size$^{\diamond}$ ↓ O^6-methylguanine adduct (oesophagus)$^{\diamond}$
Oesophagus, NMBA-treated rats [50]	Freeze-dried black raspberries	0.3	25	↓ Tumour incidence$^{\diamond}$ ↓ Tumour multiplicity ↔ Tumour volume ↓ c-Jun (preneoplastic lesions, papillomas) ↓ i-NOS (preneoplastic lesions, papillomas) ↓ COX-2 (preneoplastic lesions) ↔ COX-2 (papillomas)
Oesophagus, NMBA-treated rats [51]	Freeze-dried black raspberries	Not reported	19	↓ VEGF-C (oesophagus) ↓ Microvessel density (oesophagus)
Oesophagus, NMBA-treated rats [52]	Freeze-dried black raspberries	Not reported	7	↔Tumour incidence ↔ Tumour multiplicity ↔ Tumour volume ↑ Survival$^{\diamond}$
Oesophagus, NMBA-treated rats [53]	Freeze-dried black raspberries	Not reported	3	↑ Expression of 24 genes (oesophagus) ↓ Expression of 12 genes (oesophagus)
Oesophagus, NMBA-treated rats [54]	Freeze-dried black raspberries	Not reported	3	Restoration of 462 of 2,261 NMBA-dysregulated genes to near-normal levels (oesophagus)
Oesophagus, NMBA-treated rats [55]	Freeze-dried black raspberry powder/extract	Not reported	30	↓ Tumour number ↓ Tumour burden
Oral malignancy, DMBA-treated hamsters [56]	Freeze-dried black raspberries	Not reported	12	↔ Tumour size ↓ Tumour multiplicity† ↓ DNA adducts (cheek pouches)$^{\diamond}$

Table 1 continued

Model	Anthocyanin preparation	Anthocyanin dose (% diet, wt/wt)*	Length of treatment (weeks)*	Primary outcomes
Skin, TPA- and DMBA-treated mice [57]	Grape seed polyphenols (GSP)	Not reported Up to 30 mg GSP topical	1 dose	↓ ODC activity (skin) ↓ MPO activity (skin)
		Up to 20 mg GSP topical, twice/week	15	↓ Tumour incidence ↓ Tumour multiplicity
Skin, TPA- and DMBA-treated mice [58]	Pomegranate extract	Not reported 2 mg of extract topical, up to twice/week	Up to 30	↓ Tumour number ↓ Epidermal hyperplasia ↓ ODC expression (skin) ↓ COX-2 expression (skin) ↓ Phosphorylation of MAPKs (skin)
Skin, TPA- and DMBA-treated mice [39]	Cyanidin-3-glucoside	3.5 μM topical, twice/week	21	↓ Tumour number ↓ Tumour size
Skin, UVB-treated mice [59]	Delphinidin	1 mg topical	1 dose	↓ Cyclobutane pyrimidine dimers (skin) ↓ 8-oxo-dG (skin) ↓ apoptosis, TUNEL assay (skin)
Xenograft, NCI-H460 human lung carcinoma cells in mice [60]	Blend of berry extracts	Not reported	32 days	↓ Tumour volume
Xenograft, A549 human lung carcinoma cells in mice [39]	Cyanidin-3-glucoside	9.5 mg/kg intraperitoneal, three times/week	5	↓ Xenograft growth ↓ Metastases
Xenograft, LLC murine lung carcinoma cells in mice [61]	Black rice anthocyanins	0.5 oral gavage	30 days	↓ Tumour volume ↓ Tumour weight
Xenograft, PC3 human prostate carcinoma cells in mice [26]	Delphinidin	2 mg intraperitoneal, three times/week	12	↓ Tumour volume ↓ Bcl2 (tumour) ↑ Bax (tumour) ↓ NF-κB (tumour) ↓ Cyclin D1 (tumour) ↓ Ki-67 and PNCA (tumour)
Xenograft, Meth/A murine lymphoma cells in mice [62]	Red rice	Not reported	30 days	↑ Survival

8-oxo-dG, 8-hydroxyguanosine; B[*a*]P, benzo[*a*]pyrene; COX-2, cyclooxygenase-2; DMBA, 7,12-dimethylbenz(a)anthracene; DMH, 1,2-dimethylhydrazine; i-NOS, inducible nitrogen oxide; LLC, Lewis lung carcinoma; M_1dG, malondialdehyde DNA adduct; MAPK, mitogen-activated protein kinase; MPO, myeloperoxidase; NF-κB, nuclear factor-κB; NMBA, *N*-nitrosomethylbenzylamine; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; ODC, ornithine decarboxylase; PCNA, proliferating cell nuclear antigen; PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine; TPA, 12-*O*-tetradecanolylphorbol-13-acetate; TUNEL, terminal deoxynucleotidyl transferase mediated dUTP nick-end label

*Unless specified otherwise. Statistically significant ($P < 0.05$) at †certain doses or ‡ when all doses are combined, ◇ not statistically significant

↑ Increase, ↓ decrease, ↔ unchanged

with this notion, formation of anthocyanidins from anthocyanins has been demonstrated in the biomatrix in vivo (vide supra), however, at miniscule concentrations. Therefore, it is possible, but not likely, that the anthocyanidins mediate the pharmacological effects of their parent glycosides.

The case for anthocyanins as potential drugs

Just like anthocyanidins, anthocyanins engage anticarcinogenic mechanisms in cells in vitro exemplified by interference with cell proliferation, induction of apoptosis, cell cycle arrest and antioxidation [38]. Anthocyanins have been shown to affect targets germane to oncogenesis such as AP-1, MAPK, NF-κB, and COX-2, as illustrated by the properties of cyanidin-3-glucoside in A549 human lung tumour cells in vitro and in nude mouse bearing this tumour in vivo [39]. Mirtocyan at a concentration of 10 μg/ml inhibited the receptor tyrosine kinases EGFR, ErbB2, ErbB3, VEGFR-2 and VEGFR-3 [40]. So the case for advocating anthocyanins for drug development on mechanistic grounds is similar to that for anthocyanidins.

Like their aglycons, anthocyanins are systemically poorly available, mainly due to exquisite susceptibility towards biotransformation. Where they differ is with

Table 2 Chemoprevention pharmacodynamic studies of anthocyanins in humans

Study participants	Anthocyanin preparation	Anthocyanin dose (mg)*	Treatment duration (weeks)*	Primary outcome
Healthy volunteers [63]	Strawberries	Not reported	1 dose	↑ Serum antioxidant capacity (ORAC, FRAP, TEAC$^{\diamond}$) ↑ Urine antioxidant capacity (ORAC)
	Lyophilized red wine	Not reported	1 dose	↑ Serum antioxidant capacity (ORAC, FRAP, TEAC$^{\diamond}$) ↑ Urine antioxidant capacity (ORAC)
Healthy volunteers [64]	Freeze-dried blueberry powder	1,200	1 dose	↑ Serum antioxidant capacity (ORAC†, TEAC$^{\diamond}$)
Healthy volunteers [65]	Blackcurrant anthocyanin concentrate	3.6 mg/kg body weight	1 dose	↑ Serum antioxidant capacity (chemiluminescence system)
Healthy volunteers [66]	Freeze-dried blueberry powder	1,200	1 dose	↑ Serum antioxidant capacity (OCAR*, TAS*)
Healthy volunteers [67]	Strawberries	Not reported	1 dose	↓ Urinary NDMA
Healthy volunteers [68]	Anthocyanin-rich juice	69 (daily)	2	↔ Plasma antioxidant capacity (FRAP) ↓ Plasma malondialdehyde
Healthy volunteers [69]	Blackcurrant juice	285–600 (daily)	3	↓ Oxidative DNA damage MNBCs (Fpg sites) ↔ Oxidative DNA damage MNBCs (strand breaks, endo III sites)
	Blackcurrant anthocyanin concentrate	285–600 (daily)	3	↔ Oxidative DNA damage MNBCs (strand breaks, Fpg sites, endo III sites)
Healthy volunteers [70]	Blood orange juice	28 (daily)	3	↔ Plasma antioxidant capacity (meq of uric acid) ↔ Plasma malondialdehyde ↔ Urinary 11-dehydro-TXB_2 ↑ Lymphocyte resistance to oxidative DNA damage
Healthy volunteers [71]	Mixed fruit juice	139 (daily)	3	↓ DNA damage in MNBCs (comet assay) ↔ Plasma malondialdehyde ↑ Glutathione status$^{\diamond}$
Healthy volunteers [72]	Cranberry juice	2.1 (daily)	2	↔ Plasma antioxidant capacity (FRAP, ESR) ↔ Lymphocyte DNA damage (strand breaks, oxidised pyrimidines) ↔ Lymphocyte resistance to oxidative DNA damage ↔ Urinary 8-oxo-dG
Healthy volunteers [73]	Açai pulp/juice	Not reported	1 dose	↑ Plasma antioxidant capacity (ORAC) ↑ Urine antioxidant capacity (ORAC)†
Healthy volunteers [74]	Mixed fruit juice	177	1 dose	↑ Serum antioxidant capacity (CAP-e) ↓ Plasma malondialdehyde$^{\diamond}$
Premalignant oral lesions [22]	Freeze-dried black raspberry gel	Not reported	6	↓ Lesional grade in 41% of subjects ↓ LOH at tumour suppressor gene-associated loci
Premalignant oral lesions [75]	Freeze-dried black raspberry gel	Not reported	6	↓ Epithelial COX-2 ↓ Epithelial i-NOS$^{\diamond}$ ↓ Microvessel density in a subset of patients

Table 2 continued

Study participants	Anthocyanin preparation	Anthocyanin dose (mg)*	Treatment duration (weeks)*	Primary outcome
Barrett's oesophagus [24]	Freeze-dried black raspberry powder	Not reported	26	↔ Length of Barrett's lesions ↓ Urinary 8-Iso-PGF2 ↔ Urinary 8-oxo-dG
Haemodialysis patients [76]	Red fruit juice	Not reported	4	↓ DNA damage in MNBCs (comet assay) ↓ lipid peroxidation, NFκB DNA binding ↑ Glutathione status

8-Iso-PGF2, 8-epi-prostaglandin F2α; 8-oxo-dG, 8-hydroxyguanosine; 11-dehyro-TXB_2, 11-dehydrothromboxane B_2; CAP-e, cell-based antioxidant protection assay; COX-2, cyclooxygenase-2; Endo III, endonuclease III; ESR, electron spin resonance; Fpg, formamidopyrimidine DNA glycosylase; FRAP, ferric reducing ability assay; i-NOS, inducible nitrogen oxide; LOH, loss of heterozygosity; MNBCs, mononuclear blood cells; NDMA, *N*-nitrosdimethylamine; ORAC, oxygen radical absorbance assay; TAS, total antioxidant status; TEAC, Trolox equivalent antioxidant capacity assay

*Unless specified otherwise. Statistically significant ($P < 0.05$) at †certain time points, ◇ not statistically significant

↑ Increase, ↓ decrease, ↔ unchanged

respect to pharmaceutical properties. Anthocyanins are chemically more stable than anthocyanidins, as demonstrated under conditions of neutral pH [37], a fact which renders anthocyanins clearly superior candidates for drug development. The reason for the stability difference between aglycon and glycoside is the fact that the sugar moiety in the glycoside prevents or delays degradation of the α-diketone intermediate to the phenolic acid and the aldehyde (Fig. 2b). In human plasma cyanidin- and delphinidin-3-glucosides were stable for 4 h at room temperature, and on long-term storage at −80°C for 2 months [41].

Conclusion

Existence as a mixture of isomers and propensity to undergo extensive metabolism are two properties which militate against the suitability of either anthocyanidins or anthocyanins for development as drugs. In the case of anthocyanidins their considerable chemical instability is an additional undesirable pharmaceutical property. The situation is different for anthocyanins. They survive under biophase conditions for an acceptable period of time. They engage anticarcinogenic mechanisms consistent with chemoprevention, and their robust chemopreventive activity in preclinical models advocates further studies. Anthocyanins are clearly superior to their aglycone counterparts as candidates for drug development. Should single anthocyanins or mixtures be developed? Presently, not enough is known about differences in preclinical efficacy between individual anthocyanins to answer this question conclusively. If mixtures are to be developed, the appropriate strategy to determine anthocyanin pharmacokinetics needs careful planning. For example, must all constituents of a mixture including metabolites generated from them need to be quantitated, or is it sufficient to measure representative anthocyanins? Future studies have to resolve these questions. Standardised mixtures of anthocyanins fit for human consumption are commercially available for study now. Such mixtures tend to contain constituents of the fruit source other than anthocyanins, which may also contribute to chemopreventive efficacy.

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Conflict of interest statement None.

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